Novel CD3 epsilon humanized N-terminal epitope model for assessment of efficacy of T-cell engagers

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Background

T-cell engagers have proved to be a promising therapeutic strategy in immunotherapy, for redirecting T cells' activity against tumor cells. To facilitate the preclinical assessment of novel T-cell engagers and their translatability, we have developed an immunocompetent CD3ε N-terminal epitope humanized mouse model.

Methods

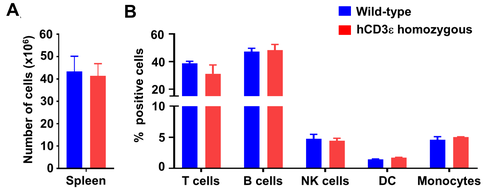
This model was developed to express the human epitope of the CD3ε chain, which is recognized by approximately 70% of the T-cell engagers (clone SP34). The rest of the extracellular domain is murine to preserve the amino acids involved in the interaction with CD3γ and CD3δ. Similarly, the transmembrane domains and the intracellular domains are murine to enable salt bridges' interaction, interaction with the CD3ζ, and the signaling into mouse cells.

Conclusions

These data suggest that the CD3ε N-terminal epitope humanized mouse model enables the assessment of efficacy and mechanism of action of T-cell engagers.
This model is currently being intercrossed with immunostimulatory humanized mouse models to provide new opportunities for assessment of bi-specific antibodies targeting the CD3 and immunostimulatory molecules. This model is the first generation of a broader program aiming at developing a pan CD3 humanized model, where the γ, δ and ε chains of the CD3 complex will be humanized. The pan CD3 humanized mice are currently being investigated for immune responses and would provide a broader tool for assessment of T-cell engagers.

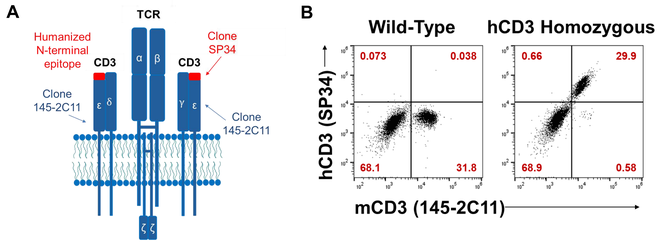
Results

1. Humanization of the epitope of CD3ε did not alter the immune cells' distribution in the hCD3ε mice



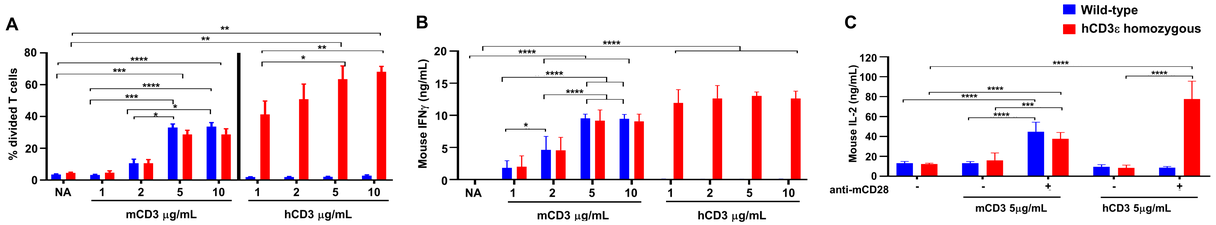
(A) Splenocytes were isolated from WT and homozygous hCD3ε mice and counted. (B) Analysis of splenic cell population gated on live cells: T cells ($CD3^+$ $CD19^-$), B cells ($CD3^-$ $CD19^+$), NK cells ($CD3^-$ $CD19^-$ $NKp46^+$), DC ($CD3^-$ $CD19^-$ $CD11b^+CD11c^+$) and monocytes ($CD3^-$ $CD19^-$ $CD11b^+$ $CD11c^-$). n=3. Statistics (Student t test) showed that cell distribution in spleen from hCD3ε is similar to WT mice. Similar data observed in bone marrow and blood (data not shown).

2. Double-positive cells are identified with similar levels from homozygous mice using clone SP34 against hCD3ε, and from WT using anti-mouse CD3ε, which binds to CD3ε, interacting with CD3γ or CD3δ chains



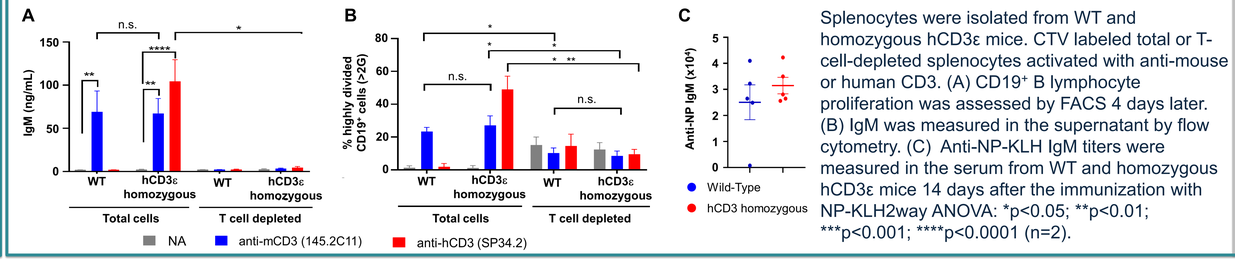
(A) Diagram showing the co-expression of human CD3ε N-terminal epitope with mouse CD3 complex. (B) Splenocytes were isolated from WT and homozygous hCD3ε mice. Expression of CD3ε N-terminal human epitope recognized by the SP34.2, as well as of mouse CD3ε, was assessed using anti-human CD3 (clone SP342) and anti-mouse CD3 (clone 145-2C11), respectively, on single live splenocytes (n=3).

3. Activation of T cells with antibodies targeting human CD3 (clone SP34) induced T cell proliferation, and production of IL-2 and IFN-γ



Splenocytes were isolated from WT and homozygous hCD3ε mice. Isolated T cells labeled with CTV and activated with various concentrations of coated anti-CD3ε (145-2C11) mouse or human (SP34) for 72h. (A) Proliferation was assessed and (B) IFN-γ levels in supernatant were quantified by ELISA. (C) IL-2 levels in the supernatant were also measured in the supernatant of cells cultured in the presence or not of anti-CD28. 2way ANOVA: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 (n=3).

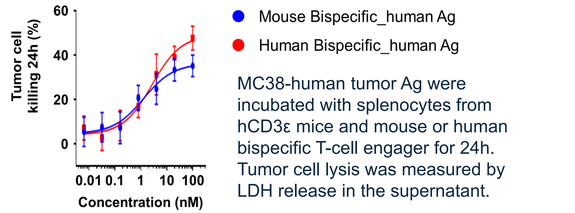
4. Production of IgM upon *in vitro* activation of T cells and *in vivo* immunization suggest a proper cooperation between T and B cells



Splenocytes were isolated from WT and homozygous hCD3ε mice. CTV labeled total or T-cell-depleted splenocytes activated with anti-mouse or human CD3. (A) $CD19^+$ B lymphocyte proliferation was assessed by FACS 4 days later. (B) IgM was measured in the supernatant by flow cytometry. (C) Anti-NP-KLH IgM titers were measured in the serum from WT and homozygous hCD3ε mice 14 days after the immunization with NP-KLH2way ANOVA: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 (n=2).

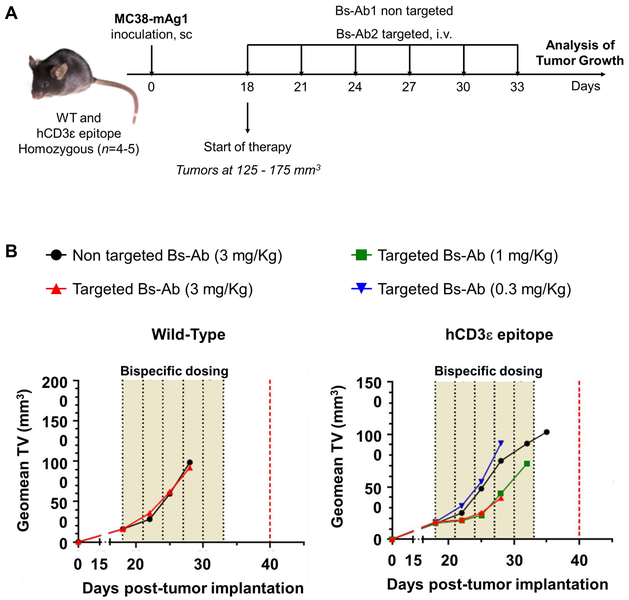
5. T-cell engagers targeting both human CD3 and a tumoral antigen induced tumor cell lysis of MC38-Ag in a concentration-dependent manner

Courtesy from Roche Innovation Center Zurich



MC38-human tumor Ag were incubated with splenocytes from hCD3ε mice and mouse or human bispecific T-cell engager for 24h. Tumor cell lysis was measured by LDH release in the supernatant.

6. A second class of T cell engagers, also targeting CD3 and a tumoral antigen, showed a dose-dependent anti-tumor effect *in vivo*



T cell engagers efficacy assessment. (A) Experimental design for *in vivo* experiments. Tumor growth curves in (B) WT and (C) hCD3ε mice.